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Heat combined with diatomaceous earth to control the confused flour beetle (Coleoptera: Tenebrionidae) in a flour mill☆

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Abstract

An alternative to methyl bromide fumigation for controlling stored-product insects in food processing facilities is to heat part or all of a facility to 50–60°C for 20–30 h. However, some equipment or structures cannot tolerate these conditions, or it is difficult or expensive to attain these high temperatures. It may be possible to reduce the temperature requirements necessary for effective control by using a desiccating dust, such as diatomaceous earth (DE), in combination with the heat treatment. The objectives of this study were to examine the combined impact of high temperature and DE on the mortality of *Tribolium confusum* (du Val) in a flour mill environment and to evaluate the effects of DE application rate on insect mortality in a mill environment during heat treatment. In areas of the mill where temperatures were in excess of 47°C, DE applications of 0.3 g/m^2 in combination with heat were no more effective than the heat treatment alone. At higher application rates, the DE was more effective. In cooler areas, adult beetles exposed to DE died sooner than insects not exposed to the insecticidal dust. These results indicate that application of DE in areas that cannot be heated to 47°C is effective for controlling *T. confusum* in a flour mill. A comparison is made with a parallel study conducted in Canada. Crown Copyright © 2001 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Diatomaceous earth; Heat treatment; *Tribolium confusum*; Flour mill

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1. Introduction

Insects in a food processing facility are a significant concern because their mere presence represents a risk that food products may become infested. Food products in a facility in which an inspector discovers stored-product insects may be deemed to be adulterated though no insects are in the food (Anonymous, 1985). Management of stored-product insects in food processing facilities, such as flour mills, can be especially difficult because of the large amount of flour dust generated during processing. Detailed sanitation programs have been developed for mills (Mills and Pedersen, 1990) but periodically additional measures are necessary to manage insect infestations.

Methyl bromide fumigation is the method of choice to manage stored-product insects infesting cereal processing facilities in North America and Europe. Because methyl bromide damages the atmospheric ozone layer, there have been steps taken to reduce its production and use. Signaturees to the Montreal Protocol on Substances that Deplete the Ozone Layer will progressively reduce their use of methyl bromide, and by 2005 only quarantine and preshipment uses will be allowed in developed countries (Anonymous, 1998). The United States plans to ban methyl bromide, except for quarantine treatment, in 2005. The reduction and elimination of methyl bromide will radically change insect control methods used by the food processing industry. Phosphine is presently the only other fumigant available for use as a structural treatment in the food processing industry but is unacceptable because it is corrosive to some metals common in processing facilities (Bond et al., 1984). Additionally, it is becoming more difficult for processing facilities situated in urban areas to use fumigant or aerosol insecticides due to municipal ordinances.

One nontoxic alternative to chemical insecticides for stored-product insect control in food processing facilities is heat treatment. Temperatures necessary to kill many species of stored-product insects have been reported (Fields, 1992) and are attainable in most food processing facilities. A heat treatment consists of heating all or part of a facility to 50–60°C and maintaining the temperature for 24–36 h. Heat treatments are not new having first been conducted in North America in the early 1900s (Dean, 1911). This method was practical in some facilities constructed primarily of wood but structural damage occurred in other wooden facilities. Modern construction using steel and concrete, however, can tolerate the high temperatures necessary for effective insect management. Imholte (1984), Heaps (1988, 1994) and Heaps and Black (1994) have reported the thermal requirements and procedures for conducting a heat treatment in a processing facility.

Although modern building construction can tolerate temperatures in excess of 50°C, some of the equipment in food processing facilities cannot. If the temperature requirements necessary for effective control could be reduced, then heat could be successfully used in these areas as well. One possible solution is to combine the heat treatment with an application of diatomaceous earth (DE). DE interferes with the insect's ability to regulate water retention by absorbing the cuticular lipids and causing death by dehydration (Ebling, 1971). In an oat mill, confused flour beetle, *Tribolium confusum* Jacquelin du Val, were completely controlled with DE after 13–22 h when temperatures reached 41°C but required 32–38 h and temperatures to rise to 47°C without the DE application (Fields et al., 1997). In laboratory tests on different formulations of DE, 100% mortality was attained after 1 d when red flour beetle, *Tribolium castaneum* (Herbst), adults were exposed to DE at 50°C for only 30 min (Dowdy, 1999a). When combined with heat, silica aerogel, which is another insecticidal dust, can reduce the time necessary to attain 100% mortality of the German cockroach, *Blattella germanica* (L.), at 43.3°C from 147 to 41 min (Ebling, 1994). DE is stable in the environment and is nontoxic to mammals (Korunic, 1998). It has a variety of commercial uses and is classified as generally recognized as safe as a food additive in the United States (Anonymous, 1991). The use, mode of action, and health aspects of DE have been recently reviewed by Korunic (1998).

The objectives of this study were to examine the combined impact of high temperature and DE on the mortality of *T. confusum* in a flour mill and to evaluate the effects of DE application rate on insect mortality in the mill environment during heat treatment.

2. Materials and methods

This study was conducted on the second and third floors of the pilot flour mill at the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, during a heat treatment on March 20–23, 1998. Each room measures 9×12 m with 4.3 m-high ceilings (Fig. 1). Hot air (60°C) was ducted into each room in the northwest corner at 2.4 m above the floor. The air was directed toward the floor through louvers on the air vents. On the second floor, a 3 m $\times$ 25 cm diameter flexible duct was attached to the air duct to direct hot air above processing equipment and toward the southeast corner. A pedestal fan was situated about 3 m from the south wall and 2 m from the east wall also to facilitate air movement into the southeast corner of the second floor. The target rate of heating was 2.5°C/h and desired maximum temperature was 50°C for 24 h.

Two areas on each floor were selected to set up the bioassay (Fig. 1). One area was located in the north end of each room near the source of hot air and the other was located in the south end of the room. We anticipated differences in the heating rate among the areas, thus allowing us to test the combined heat plus DE treatment in different conditions. A fifth area was selected in an unheated room to serve as a control. Prior to the DE application, 30×45 cm plastic sheets were taped to the floor to mask out the insecticidal dust in areas to serve as undusted controls and for an additional test using measured amounts of DE. The DE formulation selected for this study was Protect-It™ (Hedley Technologies, Mississagua, Ontario), a mixture of fresh water DE with 10% silica aerogel to enhance insecticidal efficacy (Korunic and Fields, 1995). It is a buff-colored DE containing over 87% amorphous silicon dioxide, 3% Al_2O_3, 1% Fe_2O_3, <1% CaO, MgO, TiO_3, P_2O_3, and 3–6% moisture content. Crystaline silica content is <1%. The median particle size is 5.4 μm with 20% of the particles having a diameter exceeding 12 μm. Brunauer-Emmet-Teller (BET) surface area measured by nitrogen absorption (Brunauer et al., 1938) is 10–20 m^2/g, specific gravity is 0.20, and pH is between 5.5 and 5.7 in a 10% aqueous slurry. A Flowmaster Power Duster (Model 1907, Root Lowell Company, Lowell, Michigan) made the DE application to the two rooms of the flour mill. The surface area of each room was 102 m^2 and a total of 300 g of DE was used for each room. The unheated room was not treated.

The desired rate of application was 2 g/m^2. To estimate the amount of DE applied, 9 preweighed 78.5 cm^2 plastic Petri dishes were taped to the floor in each of the four test areas. An additional 12 dishes were placed at other locations on each of the floors to estimate the deposition

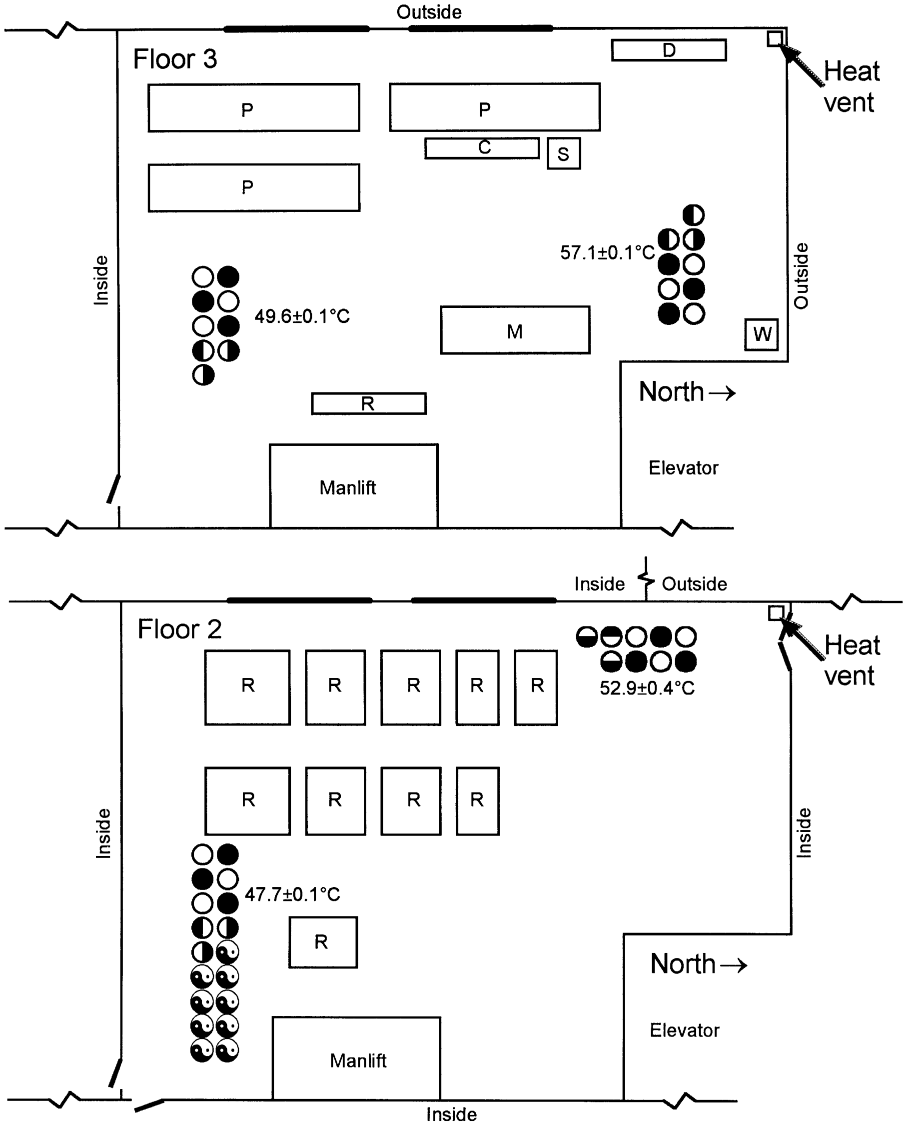

Fig. 1. Room layout and experimental locations on the second and third floors of the pilot flour mill at Kansas State University: (○) not treated with diatomaceous earth; (●) treated with diatomaceous earth; (◐) half-treated with diatomaceous earth; (☯) treated with 1, 2 or 3 g/m^2 of diatomaceous earth; (C) flour collector; (D) horizontal bran duster; (M) flour mixer; (P) flour purifier; (R) roller mill; (S) germ separator; (W) weigh hopper. Maximum temperature attained during the heat treatment is indicated next to each test location.

rate throughout the room. After the DE had settled to the floor (about 2 h), the plastic Petri dishes were collected and placed individually into zip-lock plastic bags and reweighed.

The plastic sheets were carefully removed from the floor to expose the surfaces not treated with DE. Acrylonitrile–butadiene–styrene (ABS) plastic rings (15 cm in diameter, 2.5 cm in height, 0.018 cm^2 in area) were glued to the floor using Zip-A-Way™ removable sealant (Red Devil, Inc., Pryor, Oklahoma). The rings were coated with Fluon® (polytetrafluoroethylene and polyoxyethylene (10) octylphenol), and used as test arenas for beetles exposed to the heat plus DE. The rings were placed on the floor to create three DE treatment levels; untreated, DE-treated, and half treated with the midline of the ring transected by the junction of the DE-treated and untreated surfaces. There were three rings for each treatment level within each of the four locations in the mill.

In an area that had been masked with the plastic sheets adjacent to one heated replication and in the unheated control area, rings were affixed to the floor and the enclosed areas were treated with 1, 2, or 3 g/m^2 of DE. This allowed us to determine how the rate of DE application in combination with heat impacts insect mortality, as described subsequently. There were three rings for each treatment level in the heated and unheated rooms.

Adult *T. confusum* was selected for the test because they have a high tolerance to DE (McGaughey, 1972), are flightless and, thus unable to fly out of the open test rings and the species is a common pest of food processing facilities. The beetles were maintained at 34°C and 65% r.h. on a diet consisting of whole-wheat flour and 5% brewer's yeast. The rearing temperature was relatively high for this species allowing it to develop tolerance for higher temperatures. Adult beetles were placed on fresh diet to lay eggs and transferred to new diet at weekly intervals. One- to two-week old adult beetles were collected for use in this study. Adult beetles were placed in 55-ml vials with a light dusting of flour on the day prior to the test. At the mill, the beetles were sieved from the flour and placed in the rings between 15:00 and 16:00 hours on 20 March. There were 50 beetles per ring, and mortality was checked at 60 min intervals. Dead insects were removed after each inspection and those beetles that were alive at the end of the heat treatment were transferred to Petri dishes containing 1 g of diet and held at 34°C and 65% r.h. After 1 week, final mortality was determined. Effectiveness of the heat and DE treatments was determined by the duration of survival during the treatment, the temperature at time of death, and the percentage of surviving beetles at the end of the heat treatment.

Floor temperatures were taken next to each ring by using HOBO® temperature loggers (Onset Computer Corporation, Pocasset, Massachusetts) at 10 min intervals and averaged every hour. The humidity within each replicate was measured using HOBO® RH/Temp humidity and temperature loggers (Onset Computer Corporation, Pocasset, Massachusetts) also at 10 min intervals and averaged every hour. Based on the results of Fields et al. (1997) that 100% mortality was observed at 47°C, the time for the temperature to reach 47°C and the rate of temperature increase to 47°C were calculated for the four test locations within the mill.

Analysis of variance was conducted to determine differences among treatments using PROC GLM (SAS Institute, 1995) and means separation accomplished using least significant difference (LSD) (Steel and Torrie, 1980). The results of this study were compared to a similar study conducted in Peterborough, Ontario (Fields et al., 1997) by using *t*-tests calculated with PROC MIXED (SAS Institute, 1995).

3. Results

The power duster used to apply the DE on the second and third floors of the mill did not result in the desired application rate of 2 g/m^2; only 0.31 ± 0.03 g/m^2 were actually deposited. The dust took 2–3 h to settle. Some dust escaped to other floors through the manlift opening and vents on a door. Dust also adhered to walls and equipment, further reducing the amount that contacted the floor.

Just as the DE deposition rate was below the desired level, neither the desired rate of heating nor target maximum temperature were attained during the heat treatment (Fig. 2). The north end of each room reached significantly higher temperatures than did the south end (Table 1). The length of time to reach 47°C was shortest at the north end of the second floor and significantly longer at the south end of this room (Table 1). It also took significantly longer time to reach 47°C at the south end of the second floor than at the other areas. Additionally, humidity levels were different across the rooms. The relative humidity was about 30% at the beginning of the heat treatment (Fig. 2). As the air temperature increased, the humidity initially increased and eventually dropped to 20–25%. The initial increase in humidity, however, was much greater in the south end of each room than in the north end.

The beetles died more rapidly in the north end of each room (Figs. 2b and d) than in the south end (Figs. 2a and c) ($F = 2092$, df = 3, 19, $P < 0.001$). The length of time necessary to kill 100% of

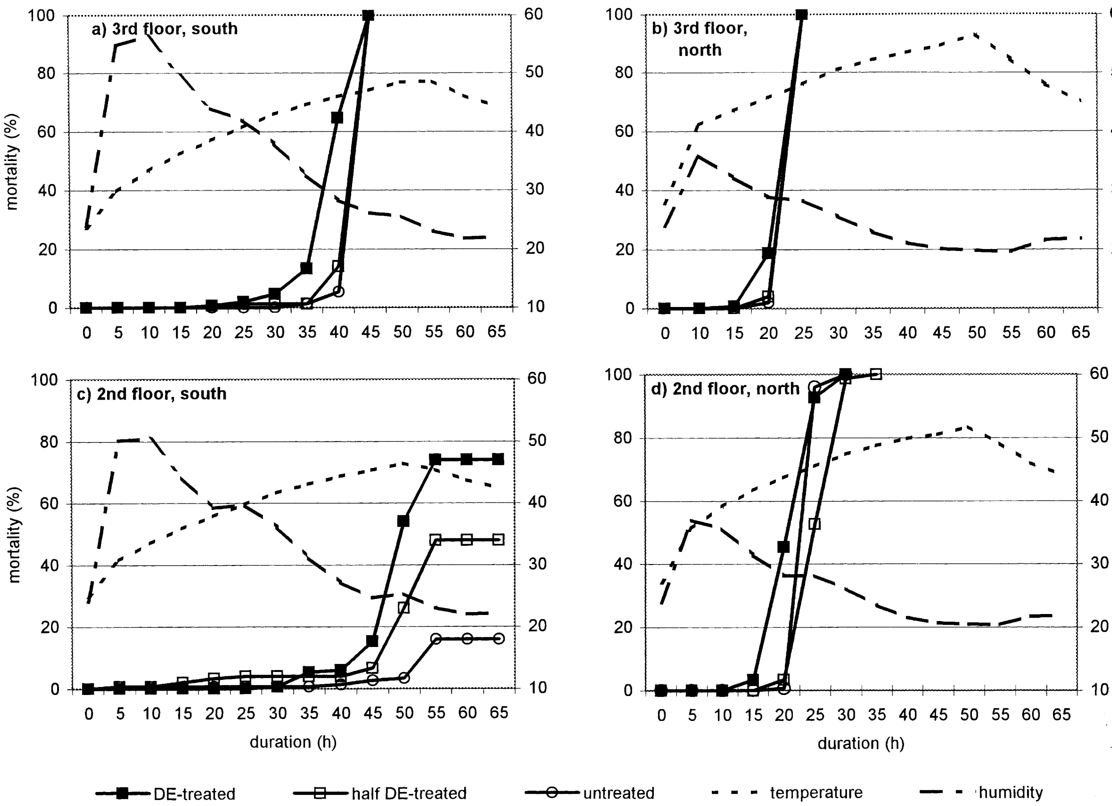

Fig. 2. Floor temperature (°C), relative humidity (%) and the cumulative percentage of mortality of *T. confusum* exposed to heat only or heat plus diatomaceous earth during a heat treatment in the pilot flour mill at Kansas State University. (a) South end of the third floor. (b) North end of the third floor. (c) South end of the second floor. (d) North end of the second floor.

Table 1
Maximum temperature, length of time to reach 47°C and rate of heating during the heat treatment (mean ± SEM) in the pilot flour mill at Kansas State University, March 1998[a]

Floor	Location	Maximum temperature (°C)	Time to reach 47°C (h)	Rate of temperature increase (Δ°C/h)
Second	South	47.7 ± 0.1 d	50.6 ± 0.1 a	0.53 ± 0.02 c
	North	52.9 ± 0.4 b	30.1 ± 1.1 c	0.92 ± 0.03 a
Third	South	49.6 ± 0.1 c	46.1 ± 0.3 b	0.60 ± 0.01 b
	North	57.1 ± 0.1 a	47.6 ± 0.3 b	0.59 ± 0.01 b
LSD		0.6	1.7	0.05
F		394	235	86
p		<0.01	<0.01	<0.01

[a] Within a column, means followed by the same letter are not significantly different ($P > 0.05$, df = 3, 32).

the beetles was 25 ± 1 h on the north end of the third floor, whereas this level of control did not occur on the south end of the second floor during the 64 h that the heat treatment was conducted. Floors 2 north and 3 south attained 100% mortality at 24 ± 1 and 43 ± 1 h, respectively (Figs. 2a and d). All insects were dead when temperatures reached between 45 and 48°C (Fig. 2).

The value of the DE application was evident only at the south end of the second floor where temperatures were lower than in the other heated areas (Fig. 2c). By the end of the heat treatment in this area, the beetles exposed to the surfaces that were fully or partially treated with DE had mortality levels of about 75 and 50%, respectively, compared to 15% for beetles exposed to heat only ($F = 6.22$, df = 2, 19, $P < 0.01$). However, 1 week after the heat treatment there was no difference in mortality between insect held with DE and heat (100%) and heat only (99%). Generally, the beetles exposed to DE began to die sooner than those not exposed to DE. In the areas that experienced higher temperatures, 100% mortality was reached at about the same time and temperature regardless of exposure to DE (Fig. 2).

Where measured amounts of DE were applied to the floor surfaces that were contained within the plastic rings, there was an obvious response in beetle mortality to the increasing dose in the heated room (south end of the second floor) but not in the unheated room (Fig. 3). The beetles exposed to the different application rates of DE began to die after about 13 h in the heated room, and mortality occurred more rapidly as the DE dosage increased (Fig. 3, Tables 2 and 3). The beetles exposed to heat without DE began to die after about 35 h of heating, which was near the time that 100% mortality was attained for the beetles exposed to the two higher doses of DE (Fig. 3a). By the end of the heat treatment, about 55% of the beetles exposed to heat only had survived (Table 2) but they were all dead after 1 week.

The effect of DE application rate on beetle mortality was not as clear in the unheated room (Fig. 3b) as it was in the heated room. First mortality occurred after about 15 h of exposure to DE and 50% mortality occurred at 29 h in the 2 g/m^2 treatment compared with about 35 and 36 h for beetles exposed to 1 and 3 g/m^2, respectively. By the end of the treatment, beetle survival was not significantly different for those exposed to DE (Tables 2 and 3). All beetles exposed to DE were dead after 1 week, and there was no mortality for beetles in the unheated room that were not exposed to the insecticidal dust.

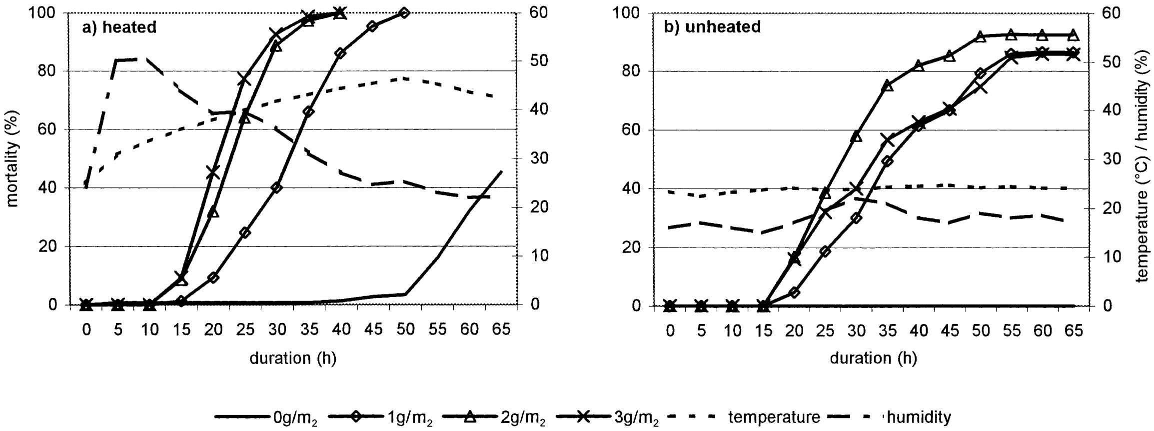

Fig. 3. Floor temperature (°C), relative humidity (%) and the cumulative percentage of mortality of *T. confusum* exposed to combinations of heat and different application rates of diatomaceous earth during a heat treatment in the pilot flour mill at Kansas State University. (a) Heated room. (b) Unheated room.

This study was designed to correspond closely with one conducted in Peterborough, Ontario (Fields et al., 1997), so that comparisons could be made between the two locations and under different environmental conditions. Because the actual amount of DE deposited using the power duster was low in Manhattan (0.31 g/m^2) compared to Peterborough (1–2 g/m^2), it is difficult to compare the results for insect mortality in the floor treatments between the two locations. Some comparisons, however, can be made where measured amounts of DE were applied by hand in heated and unheated areas.

For beetles exposed to heat only, survival was greater in Manhattan than Peterborough ($t = 3.10$, df$=4$, $P = 0.04$) (Table 2). Beetles exposed to heat plus DE, however, resulted in no survivors at both locations. The time of the first, median and last death of beetles exposed to heat only or heat plus DE was about twice as long in Manhattan as in Peterborough (Table 2). The rate of heating was much slower in Manhattan than in Peterborough, requiring more time to reach lethal temperatures. For beetles exposed to DE only, the first deaths occurred 6 or 7 h later in Manhattan than in Peterborough. For beetles exposed to 1 g/m^2 of DE, median and last deaths in unheated treatments occurred 18 and 25 h later, respectively, in Manhattan than in Peterborough (Table 2). When exposed to 3 g/m^2 of DE in the unheated test, the difference in median and last deaths was 20 and 33 h between Manhattan and Peterborough. No statistical comparison was made between Manhattan and Peterborough for the times of death because of the differences in heating rate at the two facilities. The temperature at which first, median, and last deaths occurred was not significantly different between the two locations ($F = 0.07$, df$=1, 4$, $P = 0.81$) and there was significant facility by DE treatment interaction ($F = 20.20$, df$=2, 14$, $P \leq 0.01$) (Table 2). For beetles exposed to heat only, the temperature at median mortality was about 4°C lower in Manhattan than in Peterborough ($t = 4.51$, df$=14$, $P \leq 0.01$) (Table 2). At DE rates of 1 and 3 g/m^2, however, the temperature was 3 and 1°C greater in Manhattan than in Peterborough (at 1 g/m^2: $t = 4.43$, df$=14$, $P \leq 0.01$; at 3 g/m^2: $t = 1.89$, df$=14$, $P = 0.08$) (Table 2).

Table 2
The response of *T. confusum* held in heated or unheated areas treated with or without diatomaceous earth in Manhattan or Peterborough

Response	DE application rate (g/m^2)	Manhattan[a]		Peterborough	
		Heated	Unheated	Heated	Unheated
Time at median mortality (h)	0	[b]	[b]	36 ± 2	[b]
	1	32 ± 2	35 ± 2	14 ± 1	29 ± 3
	2	23 ± 3	29 ± 1	—	—
	3	21 ± 1	36 ± 8	9 ± 1	16 ± 1
	Floor 2 south	51 ± 2	—	17 ± 1	—
	Flour 2 north	20 ± 1	—	—	—
	Floor 3 south	39 ± 1	—	—	—
	Floor 3 north	23 ± 1	—	—	—
Temperature at median mortality (°C)	0	[b]	—	46 ± 1	—
	1	43 ± 1	—	40 ± 1	—
	2	40 ± 1	—	—	—
	3	39 ± 1	—	38 ± 1	—
	Floor 2 south	47 ± 1	—	40 ± 1	—
	Flour 2 north	44 ± 1	—	—	—
	Floor 3 south	47 ± 1	—	—	—
	Floor 3 north	47 ± 1	—	—	—
Survival at end of treatment (%)	0	55 ± 17	100 ± 0	15 ± 4	100 ± 0
	1	0 ± 0	8 ± 0	0 ± 0	16 ± 10
	2	0 ± 0	5 ± 5	—	—
	3	0 ± 0	7 ± 7	0 ± 0	0 ± 0
	Floor 2 south	10 ± 9	—	0 ± 0	+
	Flour 2 north	0 ± 0	—	—	—
	Floor 3 south	0 ± 0	—	—	
	Floor 3 north	0 ± 0	—	—	—

[a] LSD for Manhattan 0–3 g/m^2: time at median mortality (h); LSD for DE = 11, LSD for heat = ns; temperature at median mortality (°C); LSD for DE = 1; survival at the end of the treatment (%); LSD for DE = 18, LSD for heat = 30.
[b] Did not occur during the course of the heat treatment.

4. Discussion

The results of this study indicate that a heat treatment without DE can be effective for controlling *T. confusum* when adequate temperatures are attained. Temperatures were not uniform throughout the test areas, resulting in some areas not reaching lethal temperatures for adequate amounts of time. Just as important, other areas may have been overheated which may result in damage to heat-sensitive equipment. Dowdy (1999b) and Fields et al. (1997) also reported uneven temperature distribution in commercial processing facilities during heat treatments, which cannot be completely controlled by redistribution of hot air into cooler areas (Dowdy, 1999b). Distribution of hot air into cooler areas can be facilitated with the use of fans but over- and under-heating may still occur (Dowdy, 1999b).

Table 3
Analysis of variance statistics for Table 2

Response	Factor	*F*	df	*P*
Time at median mortality (h)	DE	11.5	3, 5	0.02
	Heat	6.7	1, 2	0.12
	DE × heat	2.3	2, 4	0.22
Temperature at median mortality (°C)	DE	33.8	3, 5	<0.01
	Heat	5	1, 2	0.16
	DE × heat	4.4	3, 5	0.07
Survival at the end of the treatment (%)	DE	16.7	3, 5	0.01
	Heat	3171	1, 2	<0.01
	DE × heat	16.8	2, 4	0.01

In areas that are difficult to heat to temperatures lethal to insects, an application of DE appears to be of value. Although the application of DE was below desired levels, the synergism between the heat and the DE was clearly evident on the south area of the second floor. Even if the insects are not killed during a combined heat plus DE treatment, DE offers residual value for insect control if left in place after treatment. Because of the residual activity and low mammalian toxicity, DE may be suitable for use in combination with heat in areas containing heat-sensitive equipment. It also would be useful in areas that are difficult to heat and along outside walls, windows and basement floors. As temperature increases, so does insect activity, which causes insects to come in contact with DE as they move across a treated surface. Mortality due to DE is not immediate, as we frequently expect with traditional chemical insecticides, but will occur after a period of time.

The reason for differences in the synergism of DE by heat between the work conducted in Peterborough and Manhattan is unclear and requires additional study. Although the beetles used at each location were reared at different temperatures, the LT_{50} for adult confused flour beetles reared at 30° and 34°C is the same, 18.6 ± 0.6 min, when exposed to 50°C (Dowdy, unpublished data). The most probable reason is the difference in the amount of DE deposited in each study. The amount of DE reported from the Peterborough study was 1–2 g/m^2, which is more than the amount deposited in Manhattan. Another factor to consider is relative humidity, which was <5–10% in the building during the Peterborough test compared with 20–25% in Manhattan. At 25°C, death of red flour beetles occurred more quickly under conditions of low relative humidity than in more humid conditions (Aldryhim, 1993; Korunic and Fields, 1995; Fields and Korunic, 2000). The rate of heating also may have influenced the results of these two studies. Temperatures in the Peterborough facility increased more quickly than in Manhattan which may have influenced insect mortality. The slower rate of temperature increase on the south end of the second floor may have allowed time for the beetles to produce heat shock proteins that offered protection at higher temperatures (Denlinger et al., 1991). Additional work is needed to address this issue.

In areas where the temperature was >47°C for 25 h there was no benefit gained from an application of 0.31 g/m^2 of DE. Rates of 1–2 g/m^2 may prove useful. Where less heat was applied, beetles exposed to heat plus DE died sooner than those exposed to heat alone. An application of DE would be advantageous in areas that are difficult to heat or where it is desirable to heat to lower temperatures due to the presence of temperature sensitive equipment.

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